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Environmental, irrigation and fertilization impacts on the seed quality of guayule (*Parthenium argentatum* Gray)

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ABSTRACT

Guayule is a perennial shrub that originates from the Chihuahua desert. Currently stand establishment is by transplanting seedlings. In order for guayule commercialization to be more profitable, direct seeding methods need to be developed. For direct seeding to be practical factors affecting seed quality need to be identified. Guayule seed quality is highly variable. The objective of this study was to determine the seed quality of guayule (*Parthenium argentatum* Gray) grown under various field conditions in Arizona, USA, and to determine the influence of irrigation frequency and fertilization management practices on seed quality. In experiment I guayule lines AZ-2, AZ-4, AZ-R2 and 11591 were compared at four locations in Arizona (Marana, Maricopa, Yuma Mesa and Yuma Valley). In experiment II guayule lines AZ-2 and 11591 were compared under three irrigation frequencies (40%, 60% and 80%) field capacity and fertilization at low and high levels of nitrogen, at Maricopa. Germination, embryo viability, empty achene production and achene moisture content were determined for harvested achenes. In experiment I a line × location interaction occurred for normal germination, empty achenes and achene fresh weight. Line AZ-4 had the highest germination of 59% at the Yuma Valley location. Empty achenes were the highest in Marana for line 11591 at 56%. In experiment II normal germination was affected by the line, irrigation and fertilization factors. The highest germination of 66% with line 11591, 55% at 60% irrigation and 56% at high fertilization was recorded. Empty achenes were the highest with line AZ-2 at 27%. Correlations of normal germination vs. maximum temperature, empty achenes vs. total rainfall and empty achenes vs. average wind speed were positive. Negative correlations occurred for empty achene vs. maximum temperature, normal germination vs. total rainfall and normal germination vs. average wind speed. The quality of guayule seed under both experimental conditions is severely decreased by empty achene production, which seems due to genetic variability and environmental conditions during flower bloom.

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1. Introduction

Guayule (*Parthenium argentatum* Gray) is a perennial shrub native to the Chihuahua desert and occurs on the drylands of north-central Mexico (in Coahuila, Durango, Zacatecas, San Luis Potosi and Neuvo Leon) and in the Big Bend area (Presidio, Brewster and Pecos counties) of southwest Texas (Lloyd, 1911; Hammond and Polhamus, 1965; West et al., 1991). It occurs naturally at an altitude of 600–2000 m.a.s.l. and survives on sparse annual rainfall of 250–380 mm. The temperature range for survival is between −18 and 49.5 °C and plants are tolerant of high temperature but semi-dormancy is induced by low temperature of 4 °C (Thompson and Ray, 1989). The soils are well drained and calcareous with a pH of 6–8, and have low fertility. Guayule is considered an alternative crop for production in arid and semi-arid regions of the United States (Nakayama et al., 1991), Australia (Bedane et al., 2006; Dissanayake et al., 2008) and South Africa (Milthorpe et al., 1991).

Guayule produces latex that can be processed into rubber (Artschwager, 1943) and the rubber is found to be non-allergenic when in contact with human tissues (Cornish and Siler, 1996). The latex properties include strength, elasticity and viral impermeability that make it useful in applications where durability is required and disease transmission needs to be limited, e.g. condoms, surgical gloves and catheters. Processing of guayule also produces resin for adhesives, paints and varnishes. The bagasse, which is the residual plant material that remains after latex extraction, can be used as an energy source (Campos-Lopez and Anderson, 1983). About 90% of the total biomass remains after water-based latex extraction and the residual bagasse contains compounds such as fatty

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acid triglycerides, terpenes, sesquiterpenes and waxes (Nakayama, 2005). Resin-containing bagasse can be combined with a plastic binder to produce high-density composite boards that are resistant to termite degradation (Nakayama, 2005).

Guayule seed is an achene, i.e. seed retained in dry ovary wall, with attached disk florets and subtending bracts (Lloyd, 1911). Flowering and seed-set occur under long photoperiods due to active growth when soil moisture is available, with a large flower bloom early in spring through summer, and a lesser bloom in fall (Backhaus et al., 1989). The mature fruit is dry, single seeded and produced from a double ovary of which only one develops into a seed, i.e. cypsela (Artschwager, 1943). Fertile disk florets in the center of the flowerhead, i.e. capitulum, pollinate fertile ray florets around the edge of the capitulum to produce the seeds. Each capitulum can produce up to five small achenes of about 1 mg each and numerous achenes are produced from the compound inflorescence (Hammond and Polhamus, 1965). The seed is enclosed in two seedcoats: a soft outer coat of single cell thickness, except in the vascular bundle region and a tough inner coat consisting of a one- or two-celled membrane layer of thick-walled endosperm cells (Erickson and Benedict, 1947).

Guayule seed quality is highly variable; therefore, stand establishment by direct seeding is currently not feasible to offset the cost of transplanting (Foster and Moore, 1992). Germination is affected by the natural dormancy character of the embryo and seedcoats. Freshly harvested seed quickly enters dormancy of the embryo that can last for 2 months (Hammond and Polhamus, 1965). The seedcoats contain inhibitors such as p-hydroxybenzoic acid, protocatechuic acid, p-coumaric acid and ferulic acid that maintain dormancy for 6–12 months (Naqvi and Hanson, 1982). Guayule pollen is light and sticky, making it suitable for cross-pollination by wind and insects (Artschwager, 1943). Low percentages of seed containing fully developed embryos are produced due to poor pollination and unfavorable temperature (Benedict and Robinson, 1946) that also limit the germination potential of seed.

Guayule occurs as diploids ($2n = 2x = 36$), triploids ($2n = 3x = 54$), tetraploids ($2n = 4x = 72$), and octoploids ($2n = 8x = 144$), where numerous chromosomes are involved in meiosis (chromosome reduction division) and often results in sterility. Diploid guayule reproduces sexually, whereas triploids, tetraploids and octoploids reproduce asexually by apomixis. Apomictic plants produce sexual and asexual seeds, i.e. facultative apomixis. Diplosporous apomixis occurs in guayule, since embryo sacs are produced from diploid or unreduced megaspore mother cells. Apomixis in guayule is also considered to be pseudogamous because pollination is required for endosperm to develop from the fertilization of the central nuclei by one sperm nucleus (Estilai and Ray, 1991). Guayule also displays sporophytic self-incompatibility and many plants contain supernumerary chromosomes that complicate seed viability (Thompson and Ray, 1989).

Previous studies on improving guayule seed quality have focused on overcoming seed dormancy and have yielded positive results with osmoconditioning, polyethylene glycol, light, gibberellic acid (Chandra, 1991; Dissanayake et al., 2008), and an aqueous smoke solution treatment (Bekaardt, 2002; Bekaardt et al., 2004). Recent research (Jorge and Ray, 2005) has shown that germination can be increased by removing unfilled seeds from a seed lot. Unfilled seeds can be identified through X-ray techniques, but whether this can be used commercially to improve seed lots remains to be determined (Jorge and Ray, 2005). In Australia Bedane et al. (2006) found that maximum seed quality was achieved when seed was harvested after 329 growing degree days or about 28 days after flowering. The effects on seed quality of production location, fertility levels, and irrigation frequency have not been reported for the most recently developed guayule lines in Arizona. The objective of this study was to determine the seed quality of guayule grown under different field conditions in Arizona, and to determine the influence of irrigation frequency and fertilization management practices on seed quality.

2. Materials and methods

Two experiments were conducted in Arizona, United States, in 2003 and 2004. The sites were chosen as representative of areas in Arizona where guayule is expected to be grown. Two sites in Yuma were chosen because of different soil types, with heavy clay soil at the Yuma Valley site and sandy loam soil at the Yuma Mesa site. The photoperiod for all sites was similar with 12–14 h of daylight during the seed filling period.

The line AZ-2 (PI599675, GP-9) was selected for its vigorous growth and rubber yield in 2 years, and AZ-4 (PI599677, GP-11) for its improved latex and resin content (Ray et al., 1999). Lines were developed for uniformity of plant appearance, fast growth and high latex content rubber yielding ability in four cycles of within-family selection. Seed originated from four accessions obtained from the National Center for Genetic Resources Preservation, Fort Collins, CO. Line 11591 (PI478640) is not registered, and AZ-R2 is an unreleased breeding line.

Experiment I compared lines AZ-2, AZ-4, AZ-R2 and 11591 at four locations in Arizona–Marana (32°27′40″N, 111°14′00″W, 601 m.a.s.l.), Maricopa (33°04′07″N, 111°58′18″W, 361 m.a.s.l.), Yuma Mesa (32°36′43″N, 114°38′02″W, 58 m.a.s.l.) and Yuma Valley (32°42′45″N, 114°42′18″W, 32 m.a.s.l.) (Fig. 1). A randomized complete block design with four replications was used at each location with two border rows. Each line consisted of two rows, 1 m apart and 10 m long, 36 cm between plants and 3 m between blocks. The plots at Marana, Yuma Mesa and Yuma Valley were established from transplants in May of 2002 and plots at Maricopa were established in November of 2001 from transplants. Flood irrigation was scheduled at 40% field capacity, i.e. soil moisture capacity was maintained at 40% by irrigating to 100% field capacity when moisture reached 40%. Deep neutron probes to 2 m and surface TDR (time domain reflectometer) probes to 30 cm were used to determine field moisture levels. No irrigation was applied during winter when plants were dormant.

In experiment II a split–split–split block experimental design with six replications was used. Main plots were two fertility levels (high and low), split plots were three irrigation levels (40%, 60%, and 80% of field capacity) and split–split plots were two lines (AZ-2 and 11591). Each line consisted of four rows, 1 m apart and 20 m long, 36 cm between plants and 3 m between blocks. The trial was established from transplants in April of 2002 at Maricopa comparing irrigation levels of 40%, 60%, and 80% field capacity, fertilization levels of high and low nitrogen, and lines AZ-2 and 11591. Flood irrigation was done as per experiment I to maintain each level of soil moisture. Soil nitrogen levels were determined from the 0–120 cm profile 1 month after transplanting. Average high nitrogen was at 48.81 kg ha^{-1} and low nitrogen was at 16.35 kg ha^{-1}. High fertility plots were established following 4 years of continuous alfalfa production and low fertility plots followed cotton and unfertilized sudangrass to mine available nitrogen from the soil. The sudangrass exhibited nitrogen deficiency symptoms before the experiment was started. Weather data of temperature, rainfall and wind speed for the trial period from 2003 to 2004 was obtained from The Arizona Meteorological Network website (2005).

Dried inflorescences were harvested by hand from plots during summer of 2003 and 2004. One to 2 weeks after harvest samples were run through a belt thresher and blown in an air column to remove chaff. No further selection was done to remove achenes based on color or other criteria. Standard germination tests were adapted from AOSA (2002) and performed on sample units of 50 achenes per treatment per replication since each treatment was

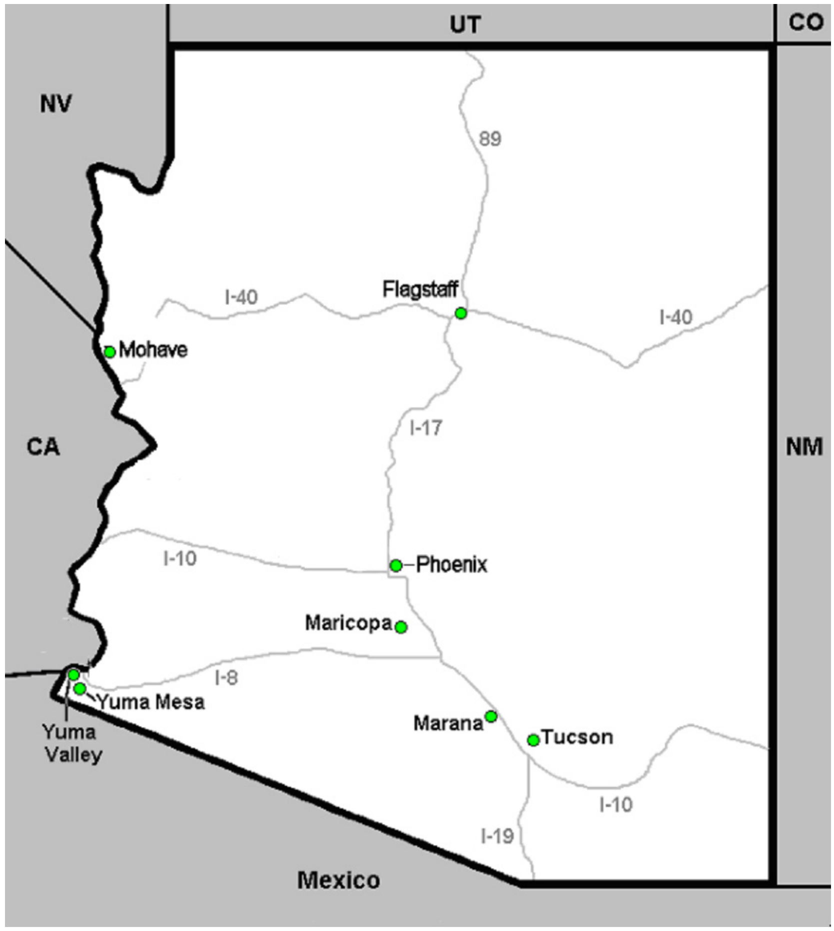

Fig. 1. Distribution of Arizona weather stations indicating guayule trial locations at Marana, Maricopa, Yuma Mesa and Yuma Valley.

already replicated in the field, i.e. experiment I: 4 replications per 4 lines per 4 locations and experiment II: 6 replications per 2 lines per 3 irrigations per 2 fertilizations. Samples were placed in plastic germination boxes on water-moistened germination paper in an incubation chamber with alternating 20 °C night (16 h) and 30 °C day (8 h) temperatures (Bakin and Baskin, 1988). No preconditioning was done prior to the achenes being placed in the germination boxes. Achenes were classified as normal, abnormal, dormant, dead and empty according to the germination results after 7 days. Normal achenes germinated to produce seedlings with both radicles and cotyledons completely developed and had the potential to develop into normal plants. Abnormal achenes germinated to produce seedlings that lacked radicles or cotyledons and were not expected to develop into normal plants. Empty achenes did not contain seeds, i.e. no embryo with endosperm had developed in the ovary wall. Dormant achenes did not germinate over the germination period, but remained firm and had the potential to germinate, whereas dead achenes had degraded and did not have the potential to germinate. Viability of dormant achenes was determined by staining with triphenyl tetrazolium chloride, while the degraded achenes were considered dead and not tested with triphenyl tetrazolium chloride. Single sections of longitudinally cut achenes of each sample were placed in a vial with 1% triphenyl tetrazolium chloride solution. The treatment was applied overnight in an oven at ±35 °C and then evaluated as per the "Tetrazolium Testing Handbook" stipulations, i.e. even staining of cotyledons with lighter or darker staining of the embryo and radicle (Perry, 1987). These were then classified as tetrazolium positive and dead achenes. Achene moisture was determined by sampling 50 achenes for each treatment and replication. The initial weight was recorded by weighing the samples and then it was dried in an oven at 100 °C. Weight remaining was recorded after 24 h and the weight difference was calculated. Moisture content was calculated as the weight difference over initial weight and then the moisture percentage was determined for each sample, i.e. on fresh weight basis.

Experiment I was analyzed as a 4 × 4 factorial for lines and locations, replicated in four blocks. Experiment II was analyzed as a 2 × 3 × 2 factorial for lines, irrigations and fertilizations, replicated in six blocks. The repeated measures for harvest were used as subplot factor in both experiments. Data were subjected to analyses of variance, test of normality and regression correlation using SAS (1990).

3. Results

Germination results presented in this study are based on total achenes including empty achenes. This may make germination percentages appear low compared to other studies which select only filled achenes for evaluation. The analyses of variance for normal germination, empty achenes and achene fresh weight among lines, locations and harvests in experiment I are shown in Table 1. Only significant results and the highest values are mentioned below. The normal germination, empty achenes and achene fresh weight variables were identified as the main components that determine the seed quality of guayule. Significant variation in the 'line × location' interaction occurred for normal germination, empty achenes and achene fresh weight. The highest normal germination at Marana was 43% for AZ-4 (Table 2). At Maricopa the highest normal germination was for 11591 at 54%, at Yuma Mesa it was 56% and at Yuma Valley AZ-4 was 59%. Empty achenes were the highest at 56% for

Table 1
Analysis of variance for normal germination, empty achenes and achene fresh weight of guayule lines AZ-2, AZ-4, AZ-R2 and 11591, from locations Marana, Maricopa, Yuma Mesa and Yuma Valley, in Arizona.

Source	Normal germination			Empty achenes		Achene fresh weight	
	DF	MS	P	MS	P	MS	P
Block	3	7.21	0.97	44.53	0.65	0.00003	<0.01*
Location	3	1077.79	<0.01*	1366.36	<0.01*	0.00037	<0.01*
Line	3	905.87	<0.01*	921.61	<0.01*	0.00059	<0.01*
Location × line	9	545.76	<0.01*	542.84	<0.01*	0.00003	<0.01*
Error (a)	45	81.67	0.66	81.46	0.85	0.000008	0.37
Harvest	1	6272	<0.01	5.28	0.83	0.00005	0.01
Harvest × location	3	725.83	0.02	1111.28	<0.01	0.00031	<0.01
Harvest × line	3	1331.25	<0.01	262.36	0.08	0.0000002	0.99
Harvest × location × line	9	158.86	0.11	168.03	0.17	0.00001	0.11
Error (b)	48	92.15		110.49		0.000007	
Corrected total	127						
Test for normality			0.87		0.99		0.09
Regression with normal germination					<0.01*		<0.01*

* Significance at $P < 0.05$.

11591 at Marana and 45% for AZ-2 at Maricopa (Table 2). At Yuma Mesa empty achenes were the highest at 46% for AZ-4 and at Yuma Valley AZ-2 was at 41%. Highest achene fresh weight at Marana was 0.033 g per 50 achenes for AZ-R2, and 0.035 g per 50 achenes for 11591 at Maricopa (Table 2). At Yuma Mesa the highest achene fresh weight was 0.041 g per 50 achenes for 11591 and 0.041 g per 50 achenes for AZ-R2 at Yuma Valley. The regression for normal germination vs. empty achenes was significant (Table 1) and indicates a negative relation with R^2 at 64% (Fig. 2). Regression between normal germination and achene fresh weight was significant (Table 1) and positive with R^2 at 30% (Fig. 2).

The analyses of variance for normal germination, empty achenes and achene fresh weight among lines, irrigations and fertilizations in experiment II are presented in Table 3. Only significant results and the highest values are mentioned below. Significant variation in the line, irrigation and fertilization occurred for normal germination. The normal germination was the highest for 11591 at 66%, 55% at 60% irrigation and 56% at high fertilization (Table 4). Significant variation in the line occurred for empty achenes. Empty achenes were at 27% for AZ-2 (Table 4). The normal germination was higher for 11591 at 66%, 55% at 60% irrigation and 56% at high fertilization (Table 4). Empty achenes were higher for the line AZ-2 at 27% (Table 4). Significant variation in the two-way interactions of fertilization × irrigation and fertilization × line occurred for achene fresh weight (Table 3). At the 40% and 60% irrigation levels with high fertilization, achene fresh weight was 0.036 and 0.034 g per 50 achenes, respectively (Table 5). With low fertilization achene fresh weight was 0.032 g per 50 achenes at the 60% irrigation level (Table 5). Achene fresh weight was the highest for line 11591 weighing 0.038 and 0.037 g per 50 achenes at the high and low fertilization levels, respectively (Table 5). The regression for normal germination vs. empty achenes was significant (Table 3), and indicates a negative relation with R^2 at 32% (Fig. 3). Regression between normal germination and achene fresh weight was significant (Table 3) and positive with R^2 at 36% (Fig. 3).

The maximum monthly temperature was similar over the trial period for all locations, with temperatures ranging from 20 to 40 °C (Fig. 4). The total monthly rainfall was different among locations (Fig. 4). The average monthly wind speeds at Marana, Maricopa, Yuma Mesa and Yuma Valley were above 8 km h^{-1} mainly dur-

Table 2
Normal germination (%), empty achenes (%) and achene fresh weight (g per 50 achenes) of guayule lines AZ-2, AZ-4, AZ-R2 and 11591, from locations Marana, Maricopa, Yuma Mesa and Yuma Valley, in Arizona.

Lines	Locations			
	Marana	Maricopa	Yuma Mesa	Yuma Valley
Normal germination				
11591	28^{bA}	54^{a}	56^{a}	56^{a}
AZ-2	38^{a}	34^{b}	34^{b}	41^{b}
AZ-4	43^{a}	41^{b}	35^{a}	59^{a}
AZ-R2	42^{a}	43^{b}	52^{a}	52^{a}
Empty achenes				
11591	56^{a}	27^{b}	28^{a}	22^{b}
AZ-2	46^{b}	45^{a}	42^{a}	41^{a}
AZ-4	35^{c}	36^{ab}	46^{a}	22^{b}
AZ-R2	38^{bc}	34^{b}	28^{b}	27^{b}
Achene fresh weight				
11591	0.030^{b}	0.035^{a}	0.041^{a}	0.040^{a}
AZ-2	0.026^{b}	0.025^{c}	0.029^{c}	0.029^{b}
AZ-4	0.032^{ab}	0.031^{b}	0.033^{b}	0.039^{a}
AZ-R2	0.033^{a}	0.033^{ab}	0.039^{ab}	0.041^{a}

[A] Means followed by different letters indicate significance at $P < 0.05$ when comparing lines within each location. Values for normal plus empty achenes may not total 100%, since abnormal, dead, and dormant achenes are not included.

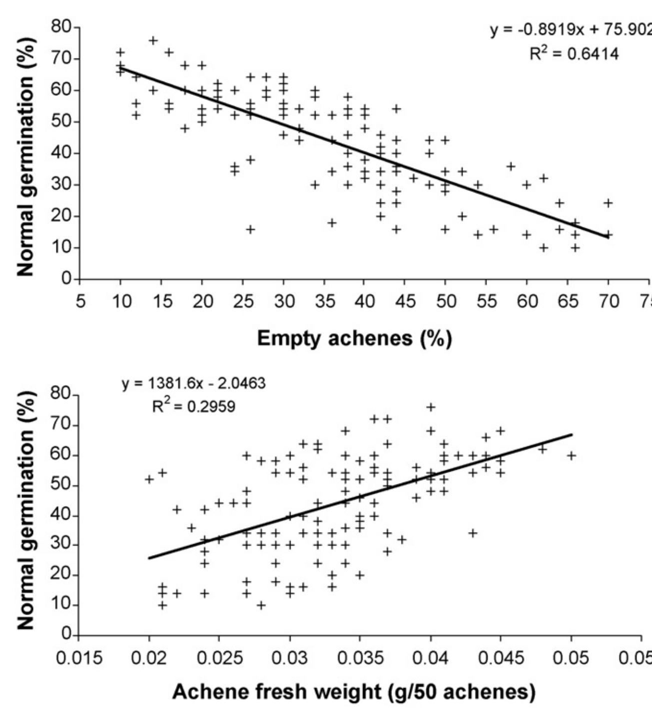

Fig. 2. Regression of normal germination vs. empty achenes and normal germination vs. achene fresh weight for experiment I. Significance level at $P < 0.05$.

Table 3
Analysis of variance for normal germination, empty achenes and achene fresh weight of guayule lines AZ-2 and 11591, from location at Maricopa, in Arizona.

Source	Normal germination			Empty achenes		Achene fresh weight	
	DF	MS	*P*	MS	*P*	MS	*P*
Block	5	27.54	0.88	62.15	0.62	0.00001	0.16
Fertilization	1	3240.71	<0.01*	97.63	0.30	0.00052	<0.01*
Irrigation	2	639.27	<0.01*	135.40	0.22	0.00030	<0.01*
Fertilization × irrigation	2	160.58	0.14	3.69	0.96	0.00004	<0.01*
Line	1	17408.63	<0.01*	5158.26	<0.01*	0.00299	<0.01*
Fertilization × line	1	86.47	0.30	5.06	0.81	0.00004	0.01*
Irrigation × line	2	35.28	0.64	52.14	0.56	0.00001	0.12
Fertilization × irrigation × line	1	311.80	0.05	110.07	0.27	0.00002	0.06
Error (a)	48	77.50	0.86	87.82	0.22	0.00001	0.08
Harvest	1	21374.06	<0.01	65.32	0.34	0.000001	0.65
Harvest × fertilization	1	3168.24	<0.01	221.30	0.08	0.00004	<0.01
Harvest × irrigation	2	457.66	0.02	586.25	<0.01	0.00001	0.10
Harvest × fertilization × irrigation	2	409.23	0.03	31.40	0.64	0.000004	0.39
Harvest × line	1	8025.56	<0.01	189.34	0.11	0.00002	0.02
Harvest × fertilization × line	1	17.80	0.68	12.52	0.68	0.00002	0.02
Harvest × irrigation × line	2	58.68	0.59	262.13	0.03	0.000005	0.24
Harvest × fertilization × irrigation × line	1	70.53	0.42	36.3	0.48	0.000000	0.89
Error (b)	51	106.05		70.93		0.000004	
Corrected total	125						
Test for normality			0.30		0.96		0.64
Regression with normal germination					<0.01*		<0.01*

* Significance at $P < 0.05$.

ing the first bloom and less during the second bloom periods (Fig. 4).

Correlations of normal germination and empty achenes with the weather data of maximum temperature, total rainfall and average wind speed are given in Figs. 5–10. There was a positive correlation ($r = 0.878$) for normal germination of line 11591 vs. maximum temperature in August and of line AZ-2 in October at $r = 0.873$ (Fig. 5). A negative correlation ($r = -0.87$ and $r = -0.753$) occurred for empty achenes with line 11591 and AZ-2 in August and October, respectively (Fig. 6). Fig. 7 indicates negative correlations of normal germination vs. total rainfall, while the correlations are positive for empty achenes vs. total rainfall in Fig. 8. Correlations are negative for normal germination vs. average wind speed (Fig. 9) and positive for empty achenes vs. average wind speed (Fig. 10).

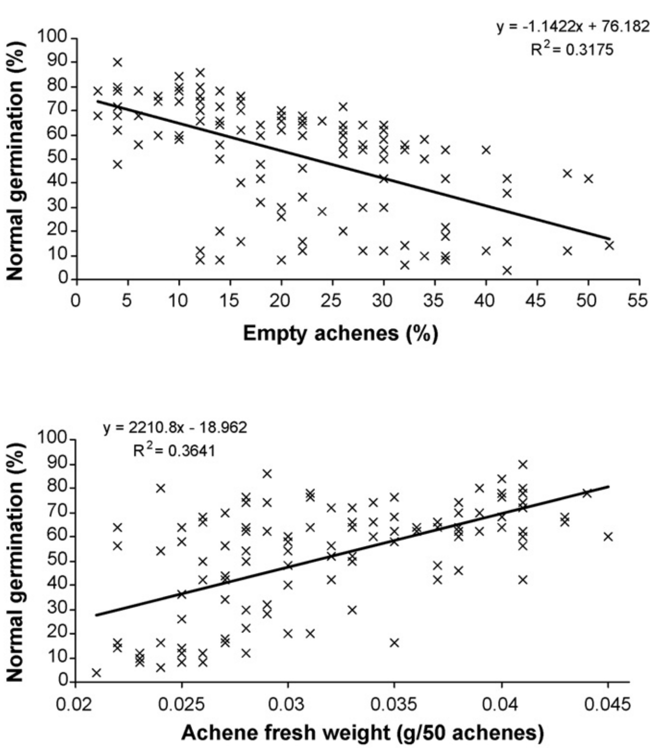

Fig. 3. Regression of normal germination vs. empty achenes and normal germination vs. achene fresh weight for experiment II. Significance level at $P < 0.05$.

Table 4
Normal germination (%) and empty achenes (%) of guayule lines AZ-2 and 11591, under 40%, 60% and 80% field capacity irrigation and at high and low fertilization levels.

	Lines		Irrigations			Fertilizations	
	11591	AZ-2	40%	60%	80%	High	Low
Normal germination	66^{a}	40^{b}	49^{b}	55^{a}	50^{b}	56^{a}	46^{b}
Empty achenes	14^{b}	27^{a}	22^{a}	20^{a}	23^{a}	21^{a}	23^{a}

Means followed by different letters indicate significance at $P < 0.05$ when comparing lines, irrigations and fertilizations, respectively.

4. Discussion

Previous studies on guayule seed germination have focused on releasing seed from dormancy and have yielded positive results with osmoconditioning, polyethylene glycol, light, gibberellic acid (Chandra, 1991; Dissanayake et al., 2008), and an aqueous smoke solution treatment (Bekaardt, 2002; Bekaardt et al., 2004). In the current study empty achene production in both experiments was the major factor that limited the germination potential of guayule seed. Empty achenes have also been noted in other recent studies (Jorge and Ray, 2005; Bedane et al., 2006; Dissanayake et al., 2008) as the major factor in low germination values for guayule seed lots.

Table 5
Fertilization × line and fertilization × irrigation interaction of guayule with respect to achene fresh weight (g per 50 achenes).

Fertilizations	Lines		Irrigations		
	11591	AZ-2	40%	60%	80%
High	0.038^{a}	0.029^{b}	0.036^{a}	0.034^{a}	0.031^{b}
Low	0.037^{a}	0.025^{b}	0.030^{b}	0.032^{a}	0.025^{c}

Means followed by different letters indicate significance at $P < 0.05$ when comparing lines within each fertilization level and in comparing irrigations within each fertilization level.

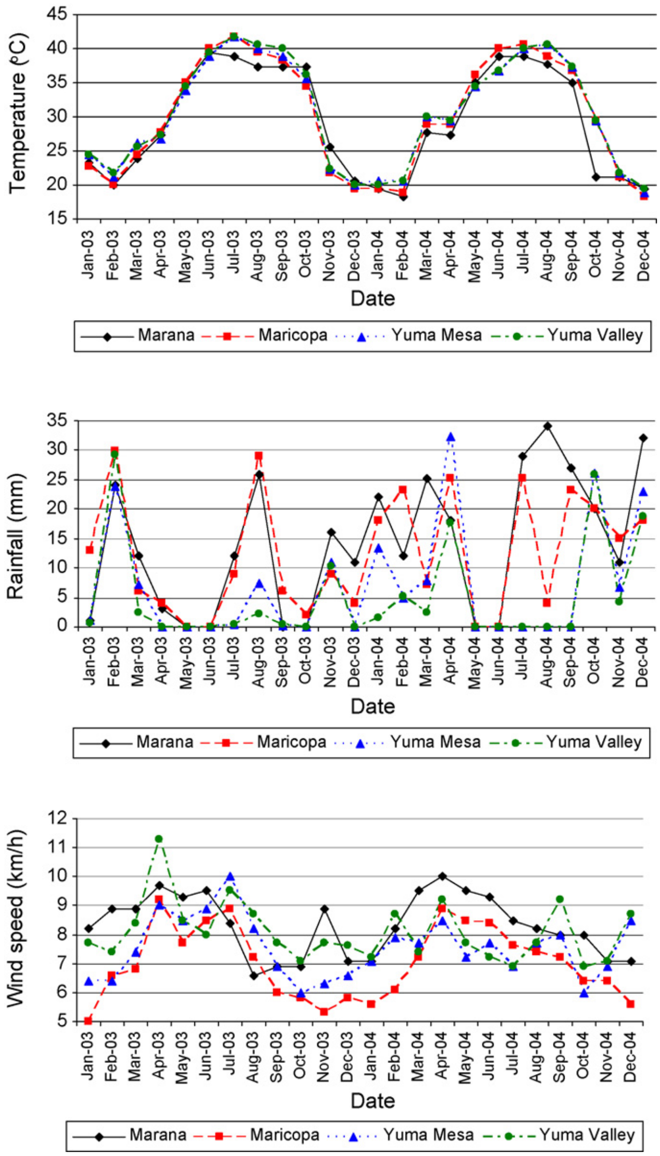

Fig. 4. Maximum monthly temperatures, total rainfall and average monthly wind speed at four guayule locations (Marana, Maricopa, Yuma Mesa and Yuma Valley) over the trial period (January 2003–December 2004).

Empty achenes can be attributed to several factors including the indeterminate flowering of guayule that results in a mixture of various levels of seed maturity in a seed lot, poor pollination that can cause poor embryo development, and/or environmental factors. More research is needed to identify the causes of empty achene formation and how to reduce the occurrence of empty achenes.

Extensive laboratory screening methods based on seed morphology, mass and X-ray analysis have been developed to predict the germination of seed (Jorge and Ray, 2005) and have proven successful to improve germination by selection for filled, heavier, gray and opaque-black seed. Application of these methods and/or others to the seed harvesting and cleaning processes would result in higher germinating seed lots.

Since seed development in guayule is due to sexual and asexual, i.e. facultative apomixis reproduction, there are no shortages of means to develop seed. Also, the polyploid character of guayule could lead to complications that may arise in meiosis, i.e. chromosome reduction division, which often results in sterility. Guayule also displays sporophytic self-incompatibility and supernumerary chromosomes that impact seed formation (Thompson and Ray, 1989).

Maximum temperature above 35 °C could have a major effect on pollination since natural pollinators such as bees are active at 20–35 °C (Copeland and McDonald, 1995). High temperature exposure of 30 °C during flowering may limit seed-set (Foster and Moore, 1992) by increasing the rate of development to maturity and early dehiscence, resulting in poor fertilization and poorly developed seed. Indications are however that normal germination was influenced positively, while empty achene production was influenced negatively by the high temperature over the flowering period.

Rainfall over the flowering period could indicate a possible limitation of seed-set as pollen is diverted from effective pollination and bee pollinators are not active during rain or on wet flowers (Copeland and McDonald, 1995). Guayule reproduces under long days with continuous flowering and seed-set from late spring in May through summer from June to August (first bloom) and into fall from September to November (second bloom) under available moisture (Whitehead and Mitchell, 1943). Negative correlation of normal germination and positive correlation of empty achene production with rainfall over the flowering period were indicated. However, significant line by location interactions also indicate that rainfall is not the only factor affecting seed-set.

Wind speeds greater than 8 km h^{-1} could limit effective pollination due to pollen displacement and reduction of bee pollinator

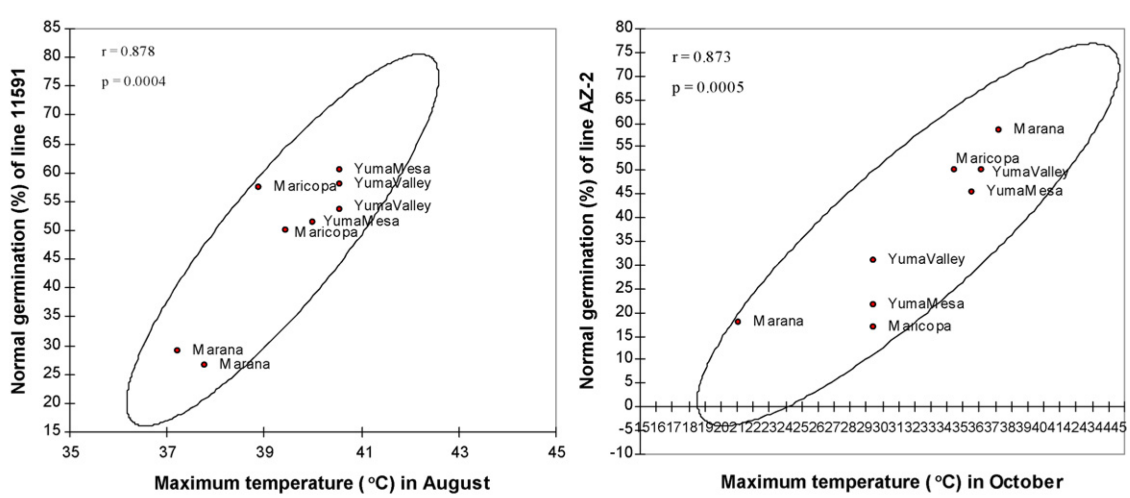

Fig. 5. Correlation of normal germination of lines 11591 and AZ-2 with the maximum temperatures in August and October, respectively, at the Maricopa, Yuma Mesa and Yuma Valley locations. Significance level at $\alpha = 0.05$.

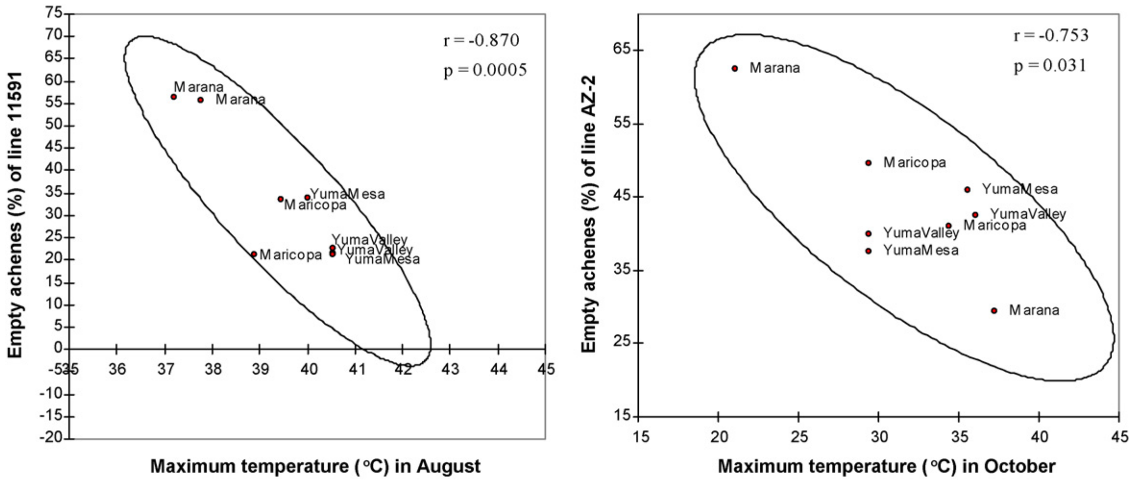

Fig. 6. Correlation of empty achenes of lines 11591 and AZ-2 with the maximum temperatures in August and October, respectively, at the Maricopa, Yuma Mesa and Yuma Valley locations. Significance level at $\alpha = 0.05$.

activity (Copeland and McDonald, 1995). Under the existing experimental conditions, wind speeds at Marana, Maricopa, Yuma Mesa and Yuma Valley were above 8 km h^{-1} mainly during the first bloom and less during the second bloom periods. The potential limitation in pollination due to wind speed and subsequent limited seed-set therefore existed at the trial locations over the trial period. Correlation of wind speed over the flowering period indicated a negative influence on normal germination and a positive influence on empty achene production.

Since pollen was observed to be abundantly shed and transferred by wind and pollinators, sufficient pollen is considered to be available for pollination. However, successful pollination cannot be

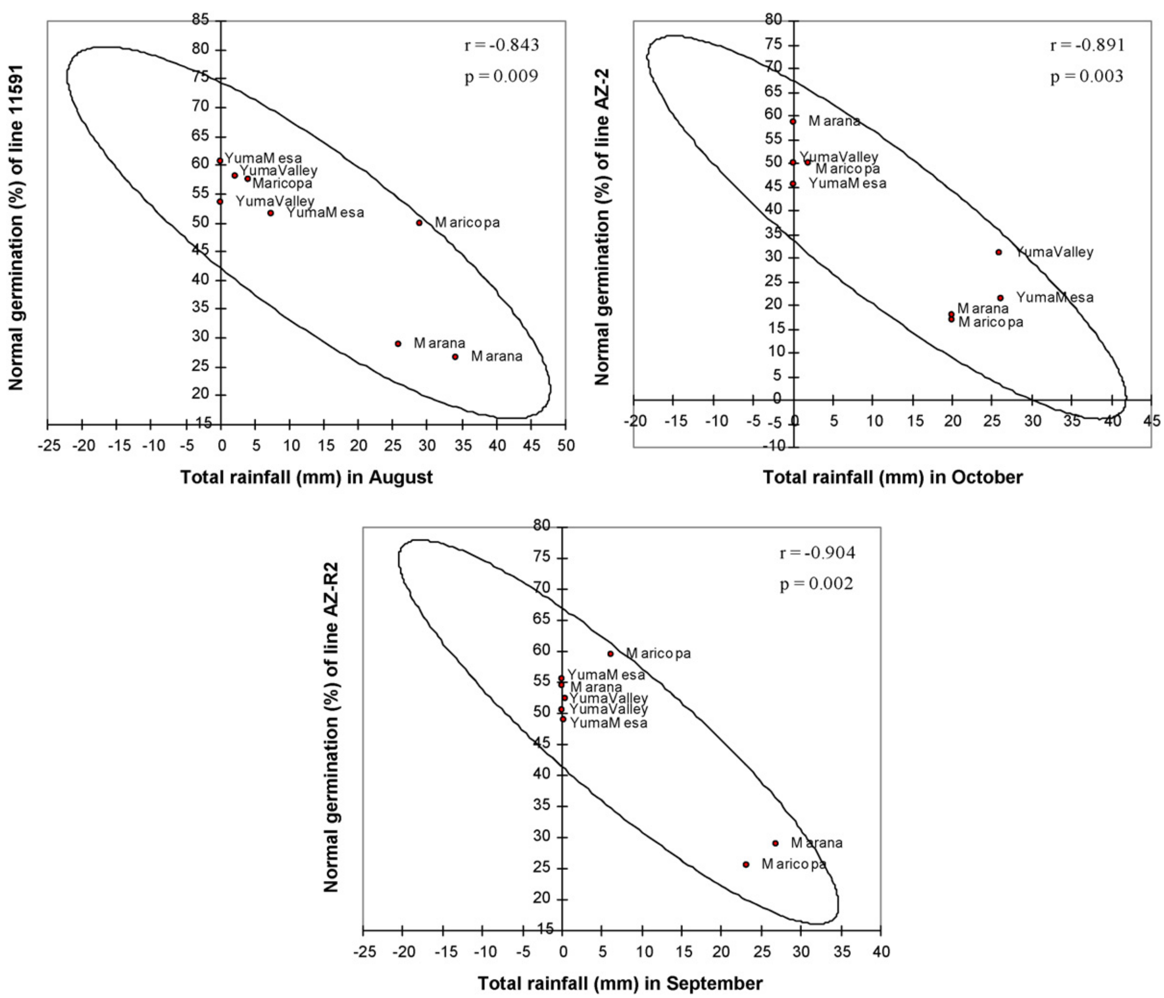

Fig. 7. Correlation of normal germination of lines 11591, AZ-2 and AZ-R2 with the total rainfall in August, October and September, respectively, at the Maricopa, Yuma Mesa and Yuma Valley locations. Significance level at $\alpha = 0.05$.

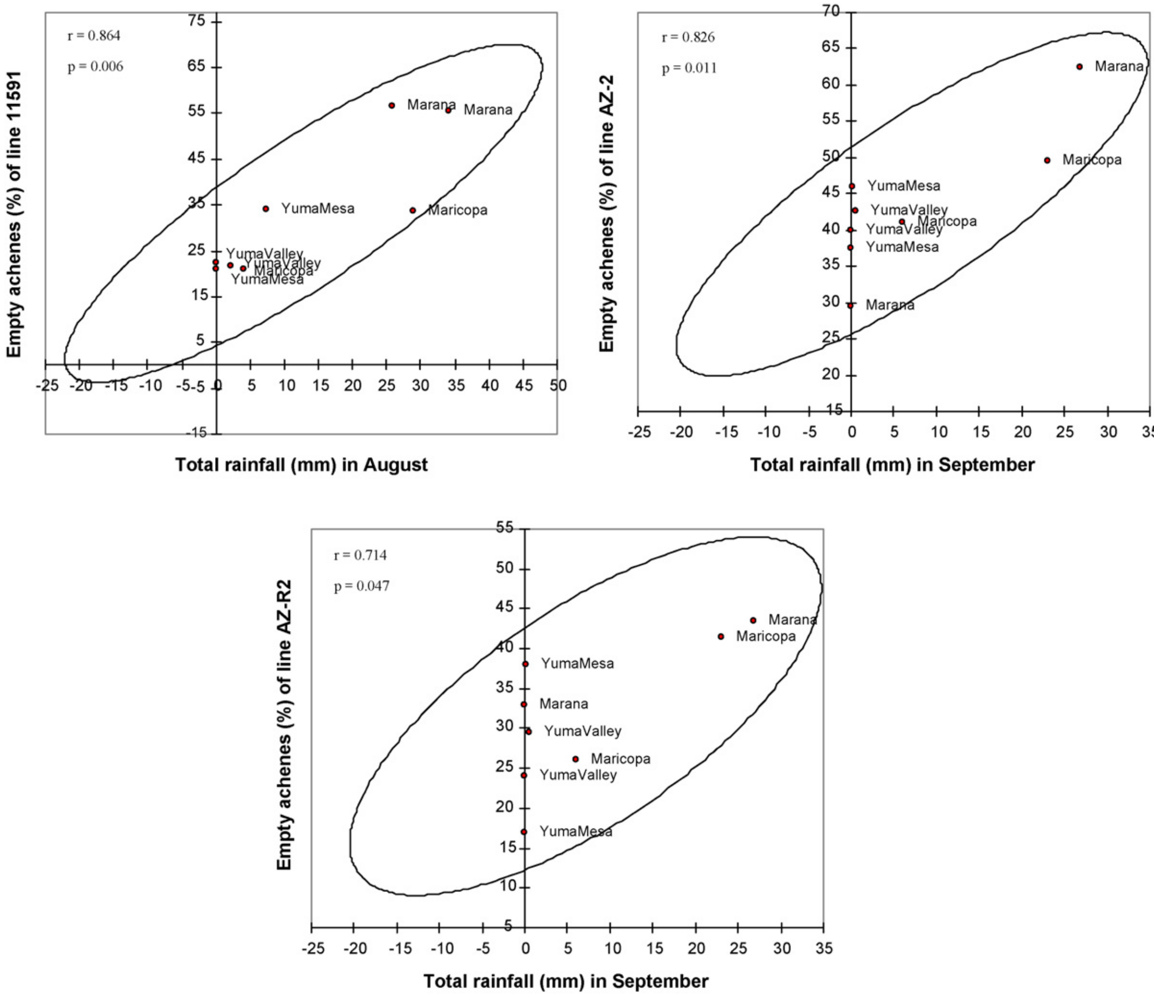

Fig. 8. Correlation of empty achenes of lines 11591, AZ-2 and AZ-R2 with the total rainfall in August, September and September, respectively, at the Maricopa, Yuma Mesa and Yuma Valley locations. Significance level at $\alpha = 0.05$.

presumed, due to excessive wind speeds and rain during flowering (Copeland and McDonald, 1995). As indicated, empty achenes are still produced so successful pollination, fertilization and subsequent seed-set does not always occur. Filled or viable guayule seeds have been reported to range from 10% to 45%. Reduced germination was caused by the development of embryos without fertilization, insect (*Lygus* spp.) feeding on flowers and succulent growing tips, dormancy imposed by the embryo and seedcoat and exposure to high temperature during seed development (Foster and Moore, 1992). The genetic variability of guayule seed production (Estilai and Ray, 1991) also still remains a factor affecting seed quality.

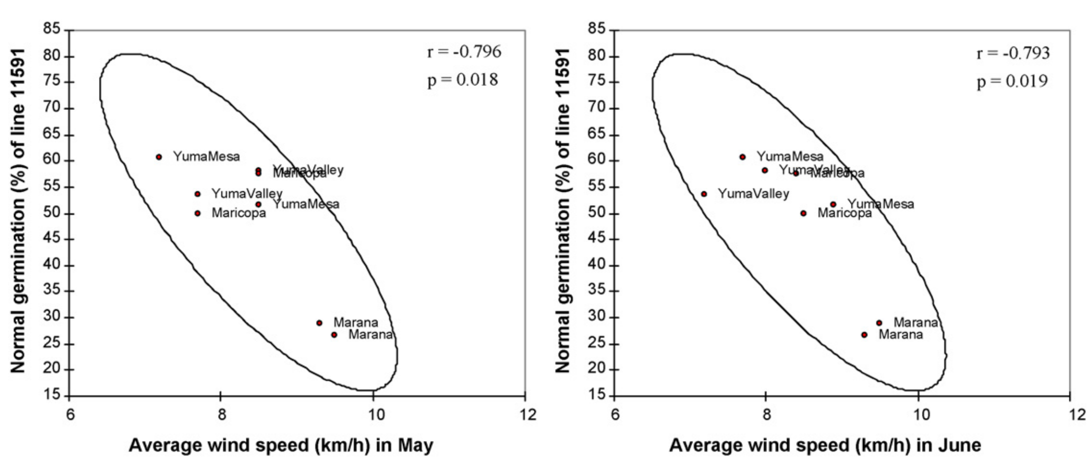

Fig. 9. Correlation of normal germination of line 11591 with the average wind speed in May and June at the Maricopa, Yuma Mesa and Yuma Valley locations. Significance level at $\alpha = 0.05$.

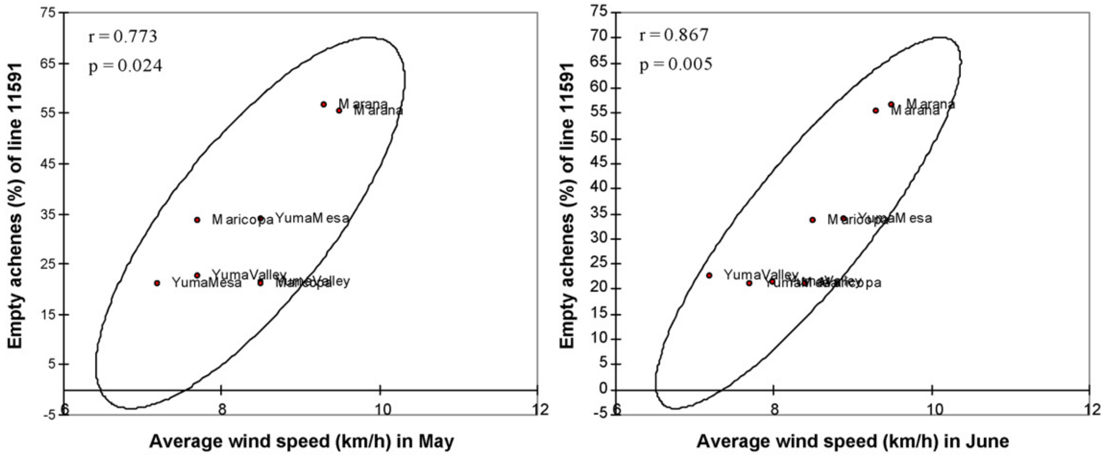

Fig. 10. Correlation of empty achenes of line 11591 with the average wind speed in May and June at the Maricopa, Yuma Mesa and Yuma Valley locations. Significance level at $\alpha = 0.05$.

5. Conclusions

The quality of guayule seed under both experimental conditions was severely decreased through empty achene production. There is not any indication that soil moisture and nutrient applications improve the seed germination potential, since no significant differences in seed germination were shown when comparing the two experiments. Indications are that high temperatures, rainfall and wind speed during flowering can influence normal germination and empty achene production. The main reason for the poor seed quality of guayule seems due to the genetic variability of guayule and is related to the environmental conditions of rainfall and winds during flowering at the experimental sites.

Results from this study and previous studies (Chandra and Bucks, 1985; Foster et al., 1999; Jorge and Ray, 2005; Bedane et al., 2006; Dissanayake et al., 2008) show that many factors can affect seed quality in guayule. Direct seeding methods must be developed for commercialization of guayule to be complete. Successful direct seeding requires that the factors affecting seed quality be identified and methods for increasing seed quality such as identifying and removing empty achenes, enhancing pollination, and seed conditioning treatments be developed.

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References

Artschwager, E., 1943. Contribution to the Morphology and Anatomy of Guayule (*Parthenium argentatum*). USDA, Washington, DC (USDA Tech. Bull. No. 842).
Association of Official Seed Analysts (AOSA), 2002. Rules for Testing Seeds. AOSA, Las Cruces, NM.
Backhaus, R.A., Higgins, R.R., Dierig, D.A., 1989. Photoperiodic induction of flowering in guayule. HortScience 24, 939–941.
Bakin, C.C., Baskin, J.M., 1988. Seeds. Ecology, Biogeography, and Evolution of Dormancy and Germination. Academic Press.
Bedane, G.M., Gupta, M.L., George, D.L., 2006. Optimum harvest maturity for guayule seed. Ind. Crops Prod. 24, 26–33.
Bekaardt, C.R., 2002. Establishment of guayule (*Parthenium argentatum* Gray). M.Sc. Thesis. Stellenbosch University, South Africa.
Bekaardt, C.R., Pieterse, P.J., Coetzee, J.H., Agenbag, G.A., 2004. The breaking of seed dormancy of guayule (*Parthenium argentatum* Gray) by treatment with gibberellic acid and smoke water solution. S. Afr. J. Plant Soil 21, 114–116.
Benedict, H.M., Robinson, J., 1946. Studies on the Germination of Guayule Seed. USDA, Washington, DC (USDA Tech. Bull. No. 921).
Campos-Lopez, E., Anderson, R.J., 1983. Natural Resources and Development in Arid Regions. Westview Press Inc., Boulder, CO.
Chandra, G.R., 1991. Osmo-conditioning improves guayule seed quality. Bioresour. Technol. 35, 141–145.
Chandra, G.R., Bucks, D.A., 1985. Improved planting quality of chemically treated guayule (*Parthenium argentatum* Gray) seeds. In: Proceedings of the 4th International Conference on Guayule Research and Development, p. 4.
Copeland, L.O., McDonald, M.B., 1995. Seed Science and Technology, 4th edn. Kluwer, London.
Cornish, K., Siler, D.J., 1996. Hypoallergenic guayule latex: research to commercialization. In: Janick, J. (Ed.), Progress in New Crops. ASHS Press, Alexandria, VA, pp. 327–335.
Dissanayake, P., George, D.L., Gupta, M.L., 2008. Direct seeding as an alternative to transplanting for guayule in southeast Queensland. Ind. Crops Prod. 27, 393–399.
Erickson, L.C., Benedict, H.M., 1947. Origin of the seed coats in guayule. J. Agric. Res. 74, 329–334.
Estilai, A., Ray, D.T., 1991. Genetics, cytogenetics and breeding of guayule. In: Whitworth, J.W., Whitehead, E.E. (Eds.), Guayule Natural Rubber, A Technical Publication with Emphasis on Recent Findings. Guayule Administrative Management Committee and USDA Cooperative State Research Service, pp. 47–92.
Foster, M.A., Moore, J., 1992. Direct-seeding techniques for guayule stand establishment in west Texas. J. Prod. Agric. 5, 163–167.
Foster, M.A., Fowler, J.L., Kleine, L.G., Puppala, N., 1999. Establishing new guayule lines by direct seeding. Ind. Crops Prod. 9, 93–100.
Hammond, B.L., Polhamus, L.G., 1965. Research on Guayule (*Parthenium argentatum*): 1942–1959. USDA, Washington, DC (USDA Tech. Bull. No. 1327).
Jorge, M.H.A., Ray, D.T., 2005. Germination characterization of guayule seed by morphology, mass and, X-ray analysis. Ind. Crops Prod. 22, 59–63.
Lloyd, F.E., 1911. Guayule (*Parthenium argentatum*), A Rubber Plant of the Chihuahuan Desert. Carnegie Institution, Washington, DC.
Milthorpe, J., Paterson-Jones, J.C., Whitworth, J.W., Abel, G., Miller, W.P., 1991. Case histories of guayule production in Australia, South Africa, and the United States. In: Whitworth, J.W., Whitehead, E.E. (Eds.), Guayule Natural Rubber, A Technical Publication with Emphasis on Recent Findings. Guayule Administrative Management Committee and USDA Cooperative State Research Service, pp. 367–421.
Nakayama, F.S., 2005. Guayule future development. Ind. Crops Prod. 22, 3–13.
Nakayama, F.S., Bucks, D.A., Gonzalez, C.L., Foster, M.A., 1991. Water and nutrient requirements of guayule under irrigated and dryland production. In: Whitworth, J.W., Whitehead, E.E. (Eds.), Guayule Natural Rubber, A Technical Publication with Emphasis on Recent Findings. Guayule Administrative Management Committee and USDA Cooperative State Research Service, pp. 145–172.
Naqvi, H.H., Hanson, G.P., 1982. Germination and growth inhibitors in guayule (*Parthenium argentatum* Gray) chaff and their possible influence in seed dormancy. Am. J. Bot. 69, 985–989.
Perry, D.A., 1987. Topographical tetrazolium test. In: Handbook of Vigour Test Methods, 2nd edn. The International Seed Testing Association, Zurich, Switzerland, pp. 57–60.
Ray, D.T., Dierig, D.A., Thompson, A.E., Coffelt, T.A., 1999. Registration of six guayule germplasms with high yielding ability. Crop Sci. 39, 300.
SAS Institute Inc., 1990. SAS/STAT User's Guide, Version 6, 4th edn. SAS Inst., Cary, NC.

The Arizona Meteorological Network, 2005. College of Agriculture & Life Sciences. University of Arizona (accessed 20.01.05) http://www.ag.arizona.edu/azmet/azdata.htm.
Thompson, A.E., Ray, D.T., 1989. Breeding guayule. Plant Breed. Rev. 6, 93–165.
West, J.E., Rodriguez, E., Hashemi, H., 1991. Biochemical evolution and species relationship in the genus *Parthenium* (Asteraceae). In: Whitworth, J.W., Whitehead, E.E. (Eds.), Guayule Natural Rubber, A Technical Publication with Emphasis on Recent Findings. Guayule Administrative Management Committee and USDA Cooperative State Research Service, pp. 17–31.
Whitehead, M.R., Mitchell, J.W., 1943. Effects of nutrient, photoperiod and night temperature on the development of guayule seeds. Bot. Gaz. 105, 14–24.